Project title:	Managing ornamental plants sustainably (MOPS)
Project number:	CP 124
Work package title:	Hot foam treatment for the control of pathogens in debris and on re-used propagation trays
Work package leader:	Erika Wedgwood
Report:	Annual report, December 2014
Previous report:	None
Key staff:	Erika Wedgwood Steven Richardson Harriet Roberts
Location of work:	ADAS Boxworth Battlegate Road Cambridge CB23 4NN
Date work commenced:	11 March 2014
Date work completed (or expected completion date):	31 October 2014 (31 October 2015 in Year 2)

DISCLAIMER

The results and conclusions in this report are based on an investigation conducted over a one-year period. The conditions under which the experiments were carried out and the results have been reported in detail and with accuracy. However, because of the biological nature of the work it must be borne in mind that different circumstances and conditions could produce different results. Therefore, care must be taken with interpretation of the results, especially if they are used as the basis for commercial product recommendations.

AUTHENTICATION

We declare that this work was done under our supervision according to the procedures described herein and that the report represents a true and accurate record of the results obtained.

Erika Wedgwood
Pathologist
ADAS UK Ltd

Signature Date 16 December 2014

[
Report authorised by:

John Atwood
Project Leader
ADAS

Signature Date 16 December, 2014

CONTENTS

GROWER SUMMARY

Headline

A new technology (Foamstream) showed good efficacy against *Pythium* and *Fusarium* and could be a valuable tool to help maintain clean and healthy plant production sites.

Background and expected deliverables

The need for surface disinfection to reduce inoculum levels of plant pathogens prior to ornamental plant production is critical for sustainable production. Some chemical disinfectants pose a risk of crop damage through vapour spread and so cannot be used when there is a growing crop in the vicinity. A treatment which can be applied close to a growing crop offers advantages in this situation. The Foamstream treatment, developed by Weedingtech as a safe non-toxic treatment for weed control in public spaces, has potential for use in reducing plant pathogen inoculum levels close to a growing crop with little risk of crop damage.

The Foamstream technique of hot foam treatment (See: www.Weedingtech.com), is currently being marketed for weed control in amenity areas. It may allow disinfection without the use of harsh chemicals of the various plant pathogens that are found in used growing media, and root/stem debris and that collects on benches and standing areas, or are spread via re-use of contaminated containers. Foamstream produces wet heat >80°C which is maintained under a natural foam insulation created by palm oils added to the heated water. The process has full organic approval and is non-polluting when used around water courses. Many fungal plant pathogens are killed by temperatures of 50-56°C, dependent on duration of exposure. Development of Foamstream for use in propagation areas and other locations could allow disinfection during the production cycle rather than waiting to the end of production because of the risk of plant susceptibility to vapours which can arise from some disinfectants.

The objectives of this work were:

- To determine if hot foam treatment will kill *Fusarium* and *Pythium* on materials used in horticultural plant production
- To determine if hot foam treatment to root debris with root rot will kill *Phytophthora* and *Pythium*
- To determine the safety of hot foam treatment to materials used in horticultural plant production

Summary of the work and main conclusions

Foamstream equipment delivers a sheet of foam from a 260 mm wide nozzle and is passed over a surface at a speed of one to two seconds. This equipment was used to evaluate the efficacy of the hot foam blanket against two common pathogens found in debris on woven ground cover and other nursery surfaces. Both artificially infested woven ground cover and infested plant roots were treated.

Pythium sp. and *Fusarium* sp. inoculum was cultured on potato dextrose agar (PDA). Plugs cut from the colonies were put on squares of woven ground-cover material, one per 90 mm diameter PDA plate. Cultures were incubated for three weeks until the hyphae had grown across and through the material and resting spores had formed within the mycelium. The infested squares of woven ground cover were doused with the Foamstream treatment for one to two seconds. *Fusarium* was also given a 10 second treatment. Untreated controls involved treating infested squares with cold water instead of the hot foam. Treatments were replicated four times. The squares were dried before plating onto agar to re-isolate.

A second test aimed to investigate whether hot foam could kill *Pythium* and *Phytophthora* spp. in raspberry root debris. The freshly harvested infested roots were scattered onto a tray of woven groundcover and Foamstream was applied for one to two seconds and an experimental time of 10 seconds. This was replicated four times with cold water controls. The roots were then plated onto agar to determine pathogen survival.

Finally, Foamstream was applied to a range of horticultural materials including pots, seed trays, polythene and expanded polystyrene to assess for thermal damage.

Agar plates containing the treated groundcover were assessed for the presence of the target pathogen and/or any other microbial contamination after three and six days incubation. Plates with roots were assessed after eight days.

The results showed the hot foam treatment was successful in killing *Pythium* after 1-2 seconds of treatment. However *Fusarium* appeared to be less susceptible to Foamstream than *Pythium* (Table 1). Foamstream was also successful at controlling *Pythium* and *Phytophthora* spp. infested raspberry roots again at both durations (Table 2).

Table 1. Results of Foamstream against *Pythium* sp. and *Fusarium* sp. on artificially infested woven ground cover

Pathogen tested and treatment given	Duration of treatment	Presence/absence (+/-) of fungal growth	
		3 days after treatment	6 days after treatment
Fusarium sp. cold water control	1-2 seconds	+	+
Pythium sp. cold water control	1-2 seconds	+	+
Fusarium sp. Foamstream	1-2 seconds	_	_
Fusarium sp. Foamstream	10 seconds	_	+
Pythium sp. Foamstream	1-2 seconds	_	_

Table 2. Results of Foamstream against *Pythium* and *Phytophthora* in raspberry roots

Treatment	Duration of treatment	Presence/absence (+/-) of fungal growth 8 days after treatment
Raspberry roots cold control	1-2 seconds	+
Raspberry roots Foamstream	1-2 seconds	_
Raspberry roots Foamstream	10 seconds	_

Foamstream was also found to be safe to use on most horticultural materials tested. However, some damage was observed on tunnel polythene with the plastic becoming slightly warped.

This new technology showed good efficacy against the oomycete root rot pathogen species of *Pythium* and *Phytophthora,* but a reduced level of control of the fungal pathogen *Fusarium oxysporum*. Foamstream treatment, designed to be used as a non-chemical weed control method, could be a valuable tool to help maintain clean and healthy plant production sites.

Action Points

There are no action points at present. The Foamstream equipment will be tested under commercial nursery conditions with natural infestations of plant pathogens and the practicalities of application on a larger scale will be examined.

SCIENCE SECTION

Introduction

There is a critical need to maintain an armoury of effective and crop-safe plant protection products for sustainable production of ornamental plants as older products are withdrawn or fail to gain re-registration. The increasing number of new conventional pesticides and biopesticides, either being developed or appearing on the market, presents an opportunity to evaluate their potential as novel solutions for ornamental plant protection, for example surface disinfection. Surface disinfection is required to reduce inoculum levels of plant pathogens prior to ornamental plant production. Some chemical disinfectants pose a risk of crop damage through vapour spread and so cannot be used when there is a growing crop in the vicinity. A non-toxic direct acting treatment which can be applied close to a growing crop offers advantages in this situation. The Foamstream treatment, developed by Weedingtech as a safe non-toxic treatment for weed control in public spaces, has potential for use in reducing plant pathogen inoculum levels close to a growing crop with little risk of crop damage.

The Foamstream technique of hot foam treatment (See: www.Weedingtech.com), which applies near-boiling water under foam, is currently being marketed for weed control in the amenity sector. Along with proven efficacy on weeds, mosses and potentially liverworts, it may also allow disinfection of the various plant pathogens and algae that are found in used growing media and root/stem debris that collects on benches and standing areas, or spread via re-use of contaminated containers. Foamstream produces wet heat >80°C and maintains heat (data on temperature decline is not readily available) under an insulating foam layer produced by natural foaming agents in the water. The 260 mm wide nozzle lance, connected via a hose to a hot water boiler, is slowly passed over surfaces so that after one to two seconds an area of 0.5 m^2 is covered by an insulating foam blanket which slowly dissipates over about 30 minutes. Many fungal plant pathogens are killed by temperatures of 50-56°C, dependent on duration of exposure. Development of Foamstream for use in propagation areas and other locations could allow disinfection during the production cycle rather than waiting to the end of the season because of potential plant susceptibility to vapours which can arise from some disinfectants.

In project HNS 147 the pathogenic basidiomycete fungus *Rhizoctonia solani* and the oomycete fungus-like organism *Pythium intermedium*, the seeds of hairy bittercress and pearlwort, leaf and bud nematode, and western flower thrips were treated with three methods of applying heat (hot-water treatment, dry oven and microwave). Wet heat (immersion in a water bath) was identified as the most effective method of application, with exposure to 60°C for 10 minutes capable of giving complete control of all of the organisms tested. It was suggested that this could be used via a hot-water bath or by use of steam/air blends. AHDB Horticulture Factsheet 20/08 gave details of how wet heat treatment of pots could be achieved on a nursery.

Foamstream has been evaluated by ADAS against weeds and liverworts in the AHDB Horticulture/HTA/EMT Fellowship CP 86. Some small scale test applications of the Foamstream nozzle over solid plastic plant containers and woven ground cover material indicated that there is potential for use of the hot foam on materials resistant to high temperatures such as propagation trays.

Based on this the objectives of this work were:

- To determine if hot foam treatment will kill *Fusarium* and *Pythium* on materials used in horticultural plant production
- To determine if hot foam treatment to root debris with root rot will kill *Phytophthora* and *Pythium*
- To determine the safety of hot foam treatment to materials used in horticultural plant production

Materials and methods

<u>Site details</u>

The trial was carried out at ADAS Boxworth in the designated spray tunnel. Foamstream treatments were applied by Weedingtech staff. Treatments were replicated four times.

Table 1. Test site and plot design information

Test location:	
County	Cambridgeshire
Postcode	CB23 4NN
Glasshouse* or Field	Polytunnel
Number of replicates	4

*Temperature and relative humidity settings are given in Appendix B

Treatment details

i) Treatment of infested ground-cover

Pythium irregulare and *Fusarium oxysporum* cultures were grown on potato dextrose agar (PDA). Single squares of alcohol-sterilised woven ground cover approximately 20 mm x 20 mm were placed on potato dextrose agar (PDA) in 90 mm plates. Three plugs of either *Pythium* sp. or *Fusarium* sp. colonies were placed across the surface of the material so that the end two overlapped onto the agar plate and weighed the square down. These plates were incubated lid upwards at 20°C in the dark for *Pythium* sp. and in a 16h light cycle for *Fusarium* sp. for a period of 3 weeks to allow the mycelium to cover the material.

On the treatment day, the squares were placed onto the centre of a 500 mm x 500 mm square of woven ground cover with corners stapled to form a shallow "tray" in order to contain the squares. *Pythium* and *Fusarium* spp. infested squares were placed in separate trays. The trays were doused by either the Foamstream for one to two or by cold water from a hose with a rose flowing at low pressure for the same period. Additional squares of *Fusarium* sp. contaminated ground-cover were available and so these were given 10 seconds of Foamstream treatment. Treatments were replicated four times.

Once the foam had disappeared after 45 minutes, the squares were then taken back to the laboratory in individual petri dishes and allowed to air-dry before placing each square onto a PDA plate. The plates were then incubated for 7 days and examined for the growth of either *Fusarium* or *Pythium* and a note also made of any other microbial contamination of the woven ground cover.

In addition, the agar plates which had held the ground cover squares (and from which the pathogens had then grown onto the agar) were also given a dousing with the Foamstream for two seconds and sections of the *Pythium* sp. and *Fusarium* sp. mycelium from these plates, and also cold water treated controls, were plated onto fresh agar to see if the pathogens had survived. It was noted that the Foamstream treatment did not melt the agar.

ii) Treatment of infected roots

Medium-fine roots were removed from raspberry plants which have been shown by lateral flow devices to have *Pythium* and *Phytophthora* spp. present. The growing medium was shaken off them to leave some still visible on the roots. The amount collected filled two 90 mm diameter Petri dishes.

The contents of each Petri dish was tipped out into the centre of 500 mm x 500 mm square of woven ground cover with corners stapled to form a shallow "tray" in order to contain the roots. The roots were then doused by either the Foamstream for 1-2, or 10 seconds; or by cold water for 1-2 seconds only.

The roots were collected and taken back to the laboratory and allowed to air-dry before placing a sample without surface sterilisation onto agar plates. Five plates for each treatment with six root sections were used on both PDA (to pick up a range of fungi) and a medium (P5ARP) to select for oomycetes such as *Pythium* and *Phytophthora* spp.. The plates were incubated for seven days and examined in particular for the growth of *Pythium* spp. or *Phytophthora* spp. and a note also made of any other microbial contamination of the woven ground cover.

iii) Treatment of nursery materials to look for any visible damage

Materials were selected from those in use at ADAS Boxworth (i.e. materials that had been used for horticultural trials and typical of those which would need to be cleaned by commercial growers) (Table 4 and Figure 4). Materials did not have debris on them in order to enable visual inspection. Two samples of each material were available so that samples treated by the Foamstream and by cold water could be examined together. They were placed on a gravel surface during treatment. Each of the materials were doused by the Foamstream for two seconds, or by cold water for the same period.

A temperature probe provided by Weedingtech was placed in a gravel tray treated with the Foamstream and left in the water that was produced with the foam until the foam dissipated.

Table 2. Detail of products tested

MOPS code number	Active ingredient(s)	Manufacturer	Batch number	% a.i	Formulation type
1. Cold water control	-	-	N/A	N/A	-
2. Foamstream	Natural foaming agents and water at 90°C+	Weedingtech	N/A	N/A	-

Table 3. Treatments

Product name or MOPS code number	Application timing	Dosage rate (a.i/ha)	Spray volume (L/ha)
1. Cold water control	A1	N/A	12 L/minute
2. Foamstream	A1	N/A	12 L/minute
Application timing			
A1	11/03/2014		

Table 4. Application details

Application No.	**A1**
Application date	11/03/2014
Time of day	Morning
Application method	Applied by Weedingtech using the Foamstream hand-held lance
Temperature of air (°C)	11.8
Relative humidity (%)	88.9
1. Materials tested for damage	10 L Soparco stiff thick pot (Polypropylene PP 05)
2.	Woven ground-cover material
3.	Expanded polystyrene carry-tray for pots
4.	Teku JP305014 multicell tray (brittle thin plastic)
5.	HSP Ltd gravel tray (stiff thick plastic)
6.	Half-size seed tray (stiff thick plastic, probably PP)
7.	180 cell tray (stiff thick plastic)
8.	3 L Soparco thin pot (Polypropylene PP 05)
9.	Polythene tunnel covering (Visqueen Luminance)

Target pathogens

Table 5. Target pathogens

Common name & pathogen type	Scientific Name	Infection level pre-application
Fusarium (fungal wilt)	*Fusarium oxysporum* (ADAS isolate AR06/56 ex stocks)	Inoculated agar & woven ground cover
Pythium (oomycete root rot)	*Pythium irregulare* (RHS isolate P71098.7.2008)	Inoculated agar & woven-ground cover
Phytophthora (oomycete root rot)	*Phytophthora rubi* (SCRP333 FVR11)	Medium/fine raspberry roots artificially infected with *P. rubi*.
Pythium (oomycete root rot)	*Pythium* spp. (species not identified)	Medium/fine raspberry roots naturally infected with *Pythium* spp.

Assessments

Table 6. Assessments of colony growth on agar after treatment on 11 March 2014

Assess-ment No.	Date	Growth stage	Timing of assessment post application	Assessment type
1	14/03/2014	N/A	After 3 days	Growth of *Fusarium* sp. or *Pythium* sp.
2	17/03/2014	NA	After 6 days	Growth of *Fusarium* sp. or *Pythium* sp.
3	19/03/2014	N/A	After 8 days	Growth of *Pythium* and *Phytophthora* spp. from roots (roots cultured 1 day after treatment)

Results

Control of target pests

Foamstream successfully controlled oomycete pathogens at both 1-2 seconds and 10 seconds duration by direct contact and within medium-fine roots (Table 7 and 8). *Pythium* and *Fusarium* spp. grew in all the cold water (control) treatments. Colonies typical of mainly *Pythium* spp., but also some *Phytophthora* spp., grew from the raspberry roots on both PDA and P5ARP media. The oomycetes grew out of untreated surface sterilised root tissue i.e.

from inside the cold water treated roots, indicating in the Foamstream treatment that the heat had penetrated inside the roots so that no alive pathogen was able to be isolated.

Foamstream treatment of *Fusarium oxysporum* for one to two seconds killed the pathogen on woven-ground cover. Conversely, however, slow growing mycelium (compared with the cold water treatment, there being no growth initially for three days) developed from other pieces of *F. oxysporum* contaminated ground-cover Foamstream treated for 10 seconds. Very inhibited growth of *Fusarium* sp. also developed from a square of agar cultured from agar plates that had held the woven ground-cover removed for treatment. There was no growth of *Pythium* sp. from a square of agar cut from a plate of this pathogen given a two second application of foam.

Table 7. Results of Foamstream against Pythium and Fusarium on artificially infested woven ground cover material showing growth of pathogens after transfer to agar after the treatment day.

Pathogen tested and treatment given	**Duration of treatment**	**Presence/absence (+/-) of fungal growth**	
		3 days after treatment	**6 days after treatment**
Fusarium sp. cold water control	1-2 seconds	+	+
Pythium sp. cold water control	1-2 seconds	+	+
Fusarium sp. Foamstream	1-2 seconds	_	_
Fusarium sp. Foamstream	10 seconds	_	+
Pythium sp. Foamstream	1-2 seconds	_	_

Table 8. Results of Foamstream against *Pythium* and *Phytophthora* spp.in raspberry roots

Treatment	**Duration of treatment**	**Presence/absence (+/-) of fungal growth 8 days after treatment**
Raspberry roots cold water control	1-2 seconds	+
Raspberry roots Foamstream	1-2 seconds	_
Raspberry roots Foamstream	10 seconds	_

Foamstream was found to be safe to use on almost all the horticultural materials tested. However, some damage was observed on tunnel polythene causing the plastic to become slightly warped. The probe (not calibrated) stood in hot water in the gravel tray recorded 95°C once foam delivery was stopped and had fallen to 56°C after 16 minutes.

Application

Speed of application has been highlighted as a concern, being slower than conventional spraying such as might be used for chemical disinfection of beds.

Discussion

Three days after the Foamstream treatment had been applied neither *Pythium* nor *Fusarium* had grown on any of the agar plates receiving the treated woven ground-cover. No *Pythium* sp. was retrieved from the woven groundcover after six days on agar following two seconds of treatment (10 seconds were not given). However, there was a small amount of *Fusarium* sp. regrowth visible six days after treatment on the plates where Foamstream had been applied to the woven groundcover for 10 seconds. There was also a small amount of *Fusarium* sp. mycelium growth on the culture plates inoculated with agar that had been treated with one to two seconds of Foamstream.

Neither *Pythium* nor *Phytophthora* spp. were isolated from any of the roots treated with Foamstream showing that Foamstream was successful in controlling both of these pathogens.

Slow growth of *Fusarium* sp. was recorded in particular from the culture plate taken from an agar plate that was treated with the Foamstream. It was likely that resting spores were present embedded, perhaps 5 mm deep, in the agar which possibly insulated the fungus from the heat. If the resting spores were stimulated to germinate by the heat then they would have slowly developed colonies. Work with this *Fusarium* sp. isolate within the chemical disinfectant work package of project CP124 has shown it to be much more resilient to treatment than *Pythium* spp. and so the ability of the Foamstream to control *Fusarium* spp. is particularly important. If the Foamstream treatment is able to trigger resting spore germination then control of *Fusarium* spp. might then be completed by re-treatment a few days later either with the Foamstream, or a disinfectant (potentially at a lower rate than would be needed otherwise).

The tunnel polythene became slightly warped by the Foamstream treatment, but this would not normally be laid on gravel when treated and this may have resulted in additional heat retention. Accurate temperature measurements need to be taken at intervals under the foam when further work is carried out. However, it does seem that although the water temperature probably drops after delivery, sufficient heat (which from previous work would be around 50 °C wet heat (Lole 2006)) was held under the foam for long enough for the oomycete pathogens to be killed. The practicality of Foamstream application on a larger scale, particularly the slow coverage rate compared with the use of a disinfectant sprayer over the same area, is being considered by the Weedingtech and will involve modifications to the equipment (Fraser Higson, pers. comm.).

Conclusions

Good efficacy was shown against the *Pythium* and *Phytophthora* species tested, but control of *Fusarium oxysporum* was less consistent. The hot foam caused no damage to pots, trays and woven ground cover and so could be a valuable tool to help maintain clean plant production sites.

In 2015, the Foamstream equipment will be tested under commercial nursery conditions with natural infestations of plant pathogens (of species yet to be determined). This will be combined with testing of some of the most effective chemical disinfectants from tests carried out within another section of this project.

References

Lole, M. (2006). Ornamentals: control of pests, pathogens and weed seeds on re-used plant containers.

Talbot, D. (2008). Factsheet 20/08. Wet heat treatment to sterilise pots for re-use. Horticultural Development Company.

Appendix A – Study conduct

ADAS UK Ltd are officially recognised by United Kingdom Chemical Regulations Directorate as competent to carry out efficacy testing in the categories of agriculture, horticulture. Internal quality management systems were followed for the study.

GLP compliance will not be claimed in respect of this study.

Appendix B – Meteorological data

The experiment was conducted inside a polytunnel at ADAS Boxworth.

Location of the weather station	ADAS Boxworth, Battlegate Road, Boxworth, Cambridge, CB23 4NN
Distance to the trial site	0 miles
Origin of the weather data	ADAS UK

Average conditions during the trial:

Month/period	Av temp (ºC)	Min temp (ºC)	Max temp (ºC)	Av RH (%)*	Rainfall (mm)
March	7.5	3.7	12	82.8	0.6

***protected crops only**

Weather at treatment application:

Month/period	Av temp (ºC)	Min temp (ºC)	Max temp (ºC)	Rainfall (mm)
March	7.5	0.4	11.8	0

Appendix C – Photographs

	
Figure 1. Application of Foamstream to containers and other nursery materials	Figure 2. Squares of woven ground cover inoculated with either Pythium or Fusarium mycelium, prior to treatment
	
Figure 3. Medium/fine raspberry roots infected with Pythium and Phytophthora on woven ground cover prior to treatment	Figure 4. A range of horticultural materials prior to treatment for assessment of thermal damage by Foamstream

	
Figure 5. Agar plate incubation of woven ground cover infested by Fusarium after treatment by Foamstream showing no growth (top) or cold water control showing pink Fusarium growth (bottom)	Figure 6. Agar plate incubation of woven ground cover infested by Pythium after treatment by Foamstream showing no growth (top) or cold water control showing white spreading Pythium growth (bottom)
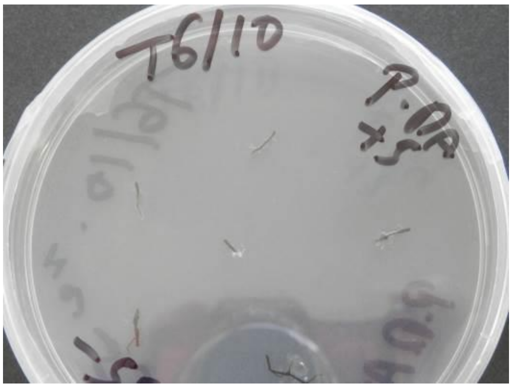	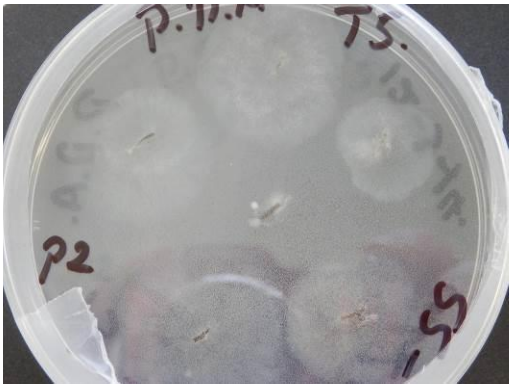
Figure 7. Agar plate incubation of raspberry roots (without surface sterilisation), treated with Foamstream (for 10 seconds, the plates for 2 seconds looked the same), showing no Oomycete or other microbial growth	Figure 8. Agar plate incubation of raspberry roots (without surface sterilisation), treated with cold water as a control, showing growth of Oomycete species

